



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 1999

**Assignment of the gene encoding the neuronal multidomain serine protease
neurotrypsin (PRSS12) to human chromosome band 4q25→q26 by in situ
hybridization**

Kozlov, S V ; Riegel, M ; Kinter, J ; Hintsch, G ; Cinelli, P ; Schinzel, A ; Sonderegger, P

DOI: <https://doi.org/10.1159/000015231>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-1078>

Journal Article

Published Version

Originally published at:

Kozlov, S V; Riegel, M; Kinter, J; Hintsch, G; Cinelli, P; Schinzel, A; Sonderegger, P (1999). Assignment of the gene encoding the neuronal multidomain serine protease neurotrypsin (PRSS12) to human chromosome band 4q25→q26 by in situ hybridization. *Cytogenetic and Genome Research*, 84(1-2):107-108.

DOI: <https://doi.org/10.1159/000015231>

Assignment¹ of the gene encoding the neuronal multidomain serine protease neurotrypsin (PRSS12) to human chromosome band 4q25→q26 by in situ hybridization

S.V. Kozlov,^a M. Riegel,^b J. Kinter,^a G. Hintsch,^a P. Cinelli,^a A. Schinzel,^b and P. Sonderegger^a

^aInstitute of Biochemistry; ^bInstitute of Medical Genetics, University of Zurich, Zurich (Switzerland)

¹ To our knowledge this is the first time this gene has been mapped.

Rationale and significance

Extracellular serine proteases, such as tissue-type plasminogen activator, plasmin, or thrombin have been implicated as important regulators of various neural functions (reviewed in Turgeon and Houenou, 1997). In the developing nervous system, serine proteases regulate cell migration, axon growth, and synapse formation (Monard et al., 1983). In the adult nervous system, a crucial involvement in synaptic plasticity associated with learning and memory operations, as well as excitotoxicity-induced neuronal death has been reported (Qian et al., 1993; Tsirka et al., 1997). During a search for novel proteases expressed in the adult neural tissue, we have recently cloned mouse neurotrypsin, a multidomain serine protease composed of a kringle domain, three scavenger receptor cysteine-rich domains, and a protease domain (Gschwend et al., 1997). Localisation of the neurotrypsin mRNA by in situ hybridisation revealed its predominant expression in the nervous system, in particular in the hippocampal formation, the amygdala, and the cerebral cortex regions exhibiting structural and functional plasticity (Gschwend et al., 1997). Here we report on the chromosome location of the neurotrypsin gene in the human genome.

Supported by the Swiss National Foundation, grant No. 32-45604.95 (M.R. and A.S.), and grant No. 31-338115.92/2 (S.K. and P.S.) and by the Théodore Ott Foundation (P.S.) S.V.K. and M.R. contributed equally to this work.

Received 18 December 1998; manuscript accepted 11 January 1999.

Request reprints from Prof. P. Sonderegger, Institute of Biochemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich (Switzerland); telephone: +41 1 635 55 41; fax: +41 1 635 68 31; e-mail: pson@bioc.unizh.ch

Materials and methods

The human neurotrypsin cDNA and genomic clones

The clone containing the homologous human neurotrypsin full-length cDNA (Genbank Accession No. AJ001531, Proba et al., 1998) was isolated from a fetal brain cDNA library (Stratagene, La Jolla CA). The cDNA insert contains 2.75 kb. Using this clone as a probe two lambda phage clones (with an approximate insert size of 16 kb and 14 kb) spanning the essential part of the genomic locus of neurotrypsin were isolated from a human genomic library prepared from the lung fibroblast cell line WI38 (Stratagene).

Fluorescence in situ hybridisation (FISH)

Metaphase chromosome spreads of a healthy donor were prepared from peripheral blood lymphocytes by standard cytogenetic procedures. Genomic and cDNA fragments were labeled with biotin-14-dUTP (Bio Nick Labeling System; Gibco BRL, Life Technologies, Gaithersburg MD). Biotin-labeled probes were detected by successive application of avidin-fluorescein-isothiocyanate (FITC) and biotinylated anti-avidin Ig (Vectashield, Vector Labs., Burlingame CA). Slides were mounted in an antifade solution (Vectashield, Vector Labs) and counterstained with DAPI. Analysis was performed with a Zeiss Axioplan epifluorescence microscope, and images were recorded by a CCD camera (Photometrics KAF, Tucson AZ), controlled with Smart Capture imaging software (Vysis, Inc., Downers Grove IL). Only metaphases displaying specific fluorescent signals at the same band position were taken into consideration.

Probe name: lambdaNT-4

Probe type: human genomic

Insert size: approximately 16 kb

Vector: lambda FIX II (Stratagene)

Proof of authenticity: Southern-blotting, partial sequencing

Results

Mapping data

Location: 4q25→q26

Number of cells examined: 42 for a karyotypically normal donor, and 13 for a donor with an insertion 46,XY,ins(6;4)(q26;q24q26)

Number of cells with specific signal: all examined cells showed the FISH hybridization signal at 4q25→q26

Most precise assignment: 4q25→q26

Location of background signals (sites with >2 signals): not observed

To confirm the FISH mapping result to 4q25→q26, further examinations with the same genomic probe were performed in a phenotypically normal donor with a 46,XY,ins(6;4)(q26;q24q26) karyotype (Schinzel et al., 1997). In this father of a malformed boy with the Rieger syndrome, a small segment of chromosome 4q25→q27 (hence the segment assumed to contain the neurotrypsin gene) is inserted into the distal long arm of one chromosome 6 (at 6q26). The hybridisation revealed one signal at 4q and the other on the distal long arm of the rearranged chromosome 6 and, thus, confirmed the location of the human neurotrypsin gene PRSS12 to 4q25→q26 (see Fig. 2).

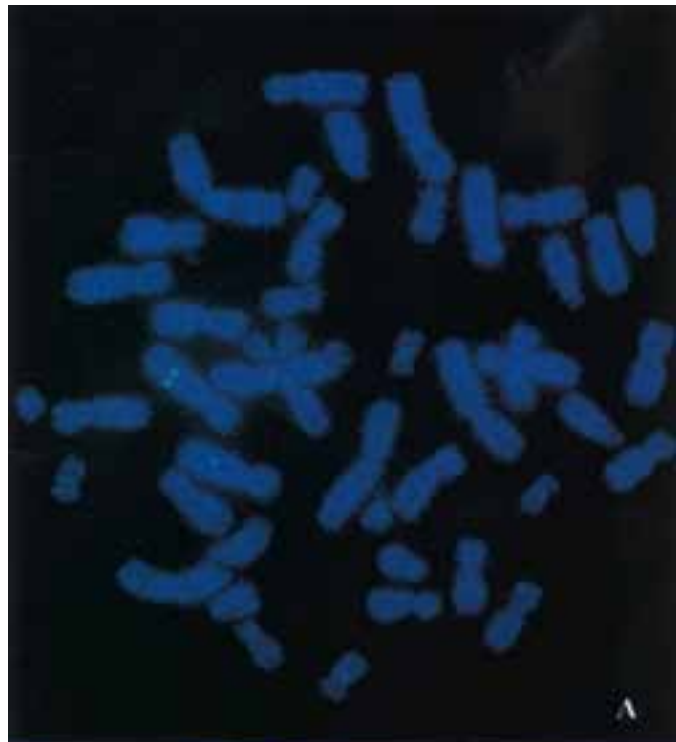


Fig. 1. Chromosomal localization of human PRSS12 to 4q25→q26. FISH with the biotinylated lambda phage clones mapping to 4q of two normal metaphases (**A** and **B**). DAPI banding of the chromosomes of metaphase **B** (**C**). The arrows indicate the region containing the signals on both chromosomes 4.

A

B



Fig. 2. Partial metaphase of a carrier of an insertion of a chromosome segment 4(q25→q27) into the distal long arm of one chromosome 6 at q26. FISH shows that the region of the human neurotrypsin gene PRSS12 maps within the inserted segment (**A**). DAPI banding of the chromosomes (**B**). 4,6 = normal homologues; del4q = deleted chromosome 4 lacking segment 4q25→q27; ins(6;4) = chromosome 6 containing segment 4q25→q27 inserted into 6q26.

References

- Gschwend TP, Krueger SR, Kozlov SV, Wolfer DP, Sonderegger P: Neurotrypsin, a novel multidomain serine protease expressed in the nervous system. *Mol Cell Neurosci* 9:207–219 (1997).
- Monard D, Niday E, Limat A, Solomon F: Inhibition of protease activity can lead to neurite extension in neuroblastoma cells. *Prog Brain Res* 58:359–364 (1983).
- Proba K, Gschwend TP, Sonderegger P: Cloning and sequencing of the cDNA encoding human neurotrypsin. *Biochim biophys Acta* 1396:143–147 (1998).
- Qian Z, Gilbert ME, Colicos MA, Kandel ER, Kuhl D: Tissue-plasminogen activator is induced as an immediate-early gene during seizure kindling and long-term potentiation. *Nature* 361:453–457 (1993).

- Schinzel A, Brecevic L, Dulty F, Baumer A, Binkert F, Largo RH: Multiple congenital anomalies including the Rieger eye malformation in a boy with interstitial deletion of (4)(q25→q27) secondary to a balanced insertion in his normal father: evidence for haplotype insufficiency causing the Rieger malformation. *J med Genet* 34:1012–1014 (1997).
- Tsirka S, Rogove AD, Bugge TH, Degen JL, Strickland S: An extracellular proteolytic cascade promotes neuronal degeneration in the mouse hippocampus. *J Neurosci* 17:543–552 (1997).
- Turgeon VL, Houenou LJ: The role of thrombin-like (serine) proteases in the development, plasticity and pathology of the nervous system. *Brain Res Brain Res Rev* 25:85–95 (1997).